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Original article (Orijinal araştırma)

Effects of artificial diets and floral nectar on parasitization performance of *Trichogramma brassicae* Bezdenko, 1968 (Hymenoptera: Trichogrammatidae)[1]

Yapay besin ve bitki nektarının *Trichogramma brassicae* Bezdenko, 1968 (Hymenoptera: Trichogrammatidae)'nin parazitleme performansına etkileri

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Summary

This study was conducted to determine whether various food resources enhanced the longevity and fecundity of the egg parasitoid *Trichogramma brassicae* Bezdenko, 1968 (Hymenoptera: Trichogrammatidae) under laboratory conditions (25°C, 65% RH, 16L:8D h photoperiod) at Laboratory of Biological Control, Department of Plant Protection, Agriculture Faculty, Namık Kemal University in 2014. Newly hatched female wasps were fed on *Ephestia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae) eggs with either honey, grape molasses and royal jelly as a main food, alone or double combination of this main foods or supplemented with resin (derived from plants), acacia nectar, *Paulownia* nectar, red tulip nectar, yellow asphodel nectar, apple syrup, liquid of *E. kuehniella* eggs or mashed *E. kuehniella* larvae. *Trichogramma brassicae*, females that were fed on honey and acacia nectar (17.47 d), honey + apple syrup (17.20 d), honey (16.93 d) and honey + *Paulownia* nectar (16.60 d) lived significantly longer than females that fed on other floral nectars and artificial diets. Females were fed on royal jelly + mashed *E. kuehniella* larvae (1.40 d) had the shortest longevity. *Trichogramma brassicae* females that were fed on honey (106.8 eggs), honey + acacia nectar (105.4 eggs), *Paulownia* nectar (103.13 eggs) parasitized significantly more hosts than females that fed on other floral nectars and artificial diets. Females fed on royal jelly were had the lowest parasitizing ability (3.33 eggs). These results showed that providing *T. brassicae* with honey, honey + acacia nectar, honey + apple syrup resulted in greater longevity and total fecundity than other food resources.

Keywords: *Trichogramma brassicae, Ephestia kuehniella,* floral nectar, food, fecundity, longevity

Özet

Bu çalışma, çeşitli besin kaynaklarının yumurta parazitoiti *Trichogramma brassicae* Bezdenko, 1968 (Hymenoptera: Trichogrammatidae)'e etkilerinin araştırılması amacıyla Namık Kemal Üniversitesi, Ziraat Fakültesi, Bitki Koruma Bölümü, Biyolojik Mücadele Laboratuvarı'nda laboratuvar koşullarında (25°C sıcaklık, %65 nem, 16:8 saat (aydınlık: karanlık) aydınlanma periyodu) 2014 yılında yürütülmüştür. Ergin dişi bireyler, değirmen güvesi *Ephestia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae) yumurtaları üzerinde ana besin (bal, üzüm pekmezi ve arı sütü) ve ara besin (reçine (bitkilerden salgılanan), akasya nektarı, çin kavağı nektarı, kırmızı lale nektarı, sarızambak nektarı, elma şurubu, *E. kuehniella* yumurta sıvısı ve ezilmiş *E. kuehniella* larvası) ve bu ana besinlerin ikili kombinasyonları ile beslenmiştir. Bal + akasya nektarı (17.47 gün), bal + elma şurubu (17.20 gün), bal + çin kavağı nektarı (16.60 gün) ile beslenen bireylerin diğer besin ve nektar ile beslenen bireylere göre daha uzun yaşadığı belirlenmiştir. En kısa ömür ise arı sütü + ezilmiş *E. kuehniella* larvası (1.40 gün) ile beslenen bireylerde görülmüştür. Çalışma sonucunda toplam parazitlenen yumurta sayısı, bal (106.8 yumurta), bal + akasya nektarı (105.4 yumurta), çin kavağı nektarı (103.13 yumurta) ile beslenen bireylerde ve belirgin olarak diğer besin ile beslenen bireylerin parazitledikleri yumurta sayılarından fazla bulunmuştur. Arı sütü ile beslenen bireylerin parazitledikleri yumurta sayısı (3.33 yumurta) en düşük olarak belirlenmiştir. Bu sonuçlar, *T. brassicae*'ye bal, bal + akasya nektarı, bal + elma şurubu verilmesinin diğer gıda kaynaklarına göre daha uzun ömür ve toplam doğurganlık sağladığını göstermiştir.

Anahtar sözcükler: *Trichogramma evanescens, Ephestia kuehniella,* bitki nektarı, besin, yumurta verimi, ömür

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Introduction

Egg parasitoids are most important parasitoids for biological control programs. *Trichogramma* species are the most widely used biological control agent. *Trichogramma* wasps are used against lepidopterans in biological control programs around the world (Li, 1994; Smith, 1996).

Food has a significant effect on parasitoid performance, such as developmental time, survival, fecundity and longevity (Hohmann et al., 1988; Fuchsberg et al., 2007; Özder & Kara, 2010, Tunçbilek et al., 2012). Parasitization performance of *Trichogramma* spp. (longevity, fecundity, adult emergence and female emergence) is known to be influenced by extrafloral nectar, pollen, honey, carbohydrate and protein (Ashley & Gonzalez, 1974; Hohmann et al., 1988; Baggen et al., 1999; Jervis et al., 2004; Lee et al., 2004, Shearer & Atanassov, 2004; Zhang et al., 2004; Wäckers, 2005; Witting-Bissinger, 2008).

Field release requires a large number of *Trichogramma* individuals (Stinner et al.,1974; Jalali & Singh, 1992). Many studies have focused on the mass rearing and storage of *Trichogramma* species (Jalali & Singh, 1992; Özpınar & Kornoşor, 1993; Özpınar, 1994; Karabörklü & Ayvaz, 2007; Yılmaz et al., 2007; Ayvaz et al., 2008).

Study on the mass rearing of parasitoids has focused primarily on food. Both longevity and fecundity of parasitoids can increase with of certain kinds of food (Ashley & Gonzalez, 1974; Özkan, 2007; Tunçbilek et al., 2012; Çınar et al., 2015) In the field parasitoids can obtain carbohydrates in homopteran honeydew, floral and extrafloral nectar (Wäckers et al., 2008). A number of studies have provided considerable evidence for increased parasitoid abundance and parasitism level when flowering plants are present (Berndt et al., 2006; Diaz et al., 2012, Masetti et al., 2010; Zhu et al., 2013).

Acacia nectar, *Paulownia* nectar, red tulip nectar, yellow asphodel nectar, honey, grape molasses and royal jelly were chosen for this study because of their floral morphology and they are widely planted in in gardens. Also, several laboratory studies have shown that hymenopteran parasitoids exhibit increased longevity and fecundity when provided with honey.

The aim of this study was to determine the effects of food sources on *Trichogramma brassicae* Bezdenko, 1968 (Hymenoptera: Trichogrammatidae) longevity, parasitism rate, progeny production and progeny sex ratio for mass rearing and in the field.

Materials and Methods

Trichogramma brassicae used in this experiment had been continuously reared on eggs of the Mediterranean flour moth, *Ephestia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae) since 1998 in the laboratory at 25 ± 1°C and 70 ± 5% RH with a 16L:8D h photoperiod. Fresh (less than 24 h old*) E. kuehniella* eggs were glued on pieces of white cardboard (2 x 4 cm) and were then placed in glass vials (7.5 x 2 cm). These eggs were offered to single *T. brassicae* females for 24 h and then were discarded. Females used in the experiments were newly emerged, mated and lacked egg laying experience. Sex ratio was calculated by using the form of the antenna to distinguish the adult females (Özder, 2006).

Eggs of *E. kuehniella* were used for rearing and as host eggs in the experiment. *Ephestia kuehniella* were reared on wheat bran. Throughout the rearing, cultures were kept in a rearing room, equipped with a control system, at 25 ± 1°C, 70 ± 5% RH. To obtained eggs, approximately 100 mated females of Mediterranean flour moth were collected from stock cultures and released in plastic jars (Özder & Kara, 2010).

Twenty-seven diet treatments were assessed (Table 1). Mature females were fed with either honey, grape molasses or royal jelly as a main food, alone or supplemented with resin (derived from plants), acacia nectar, *Paulownia* nectar, red tulip nectar, yellow asphodel nectar, apple syrup, liquid of *E. kuehniella* egg and mashed *E. kuehniella* larvae. All of the female parasitoids were fed daily until all female parasitoids died.

About 50 fresh *E. kuehniella* eggs were placed in glass vials (10 x 3 cm) and newly emerged (< 24 h) *T. brassicae* females were introduced and held for 24 h. After exposure, the adults were removed and the number of parasitized eggs counted. Fresh eggs of *E. kuehniella* were exposed to *T. brassicae* adults in glass vials until all female parasitoids died. All experiments were carried out at 25 ± 1°C and 70 ± 5% RH with a 16L:8D h photoperiod. Treatments were replicated 15 times. The number of parasitized eggs, adults and sex ratio were determined after the larval and pupal development of the parasitoids. For each sample sheet, the number of host eggs parasitized (blackened eggs) was counted daily for 5 d following exposure. To assess the effect of the diets on the longevity of *T. brassicae*, the flowers and other diets were offered simultaneously to single *T. brassicae* females. Treatments were replicated 15 times and the food sources and cardboard sheets were replaced daily until all parasitoids died. The longevity of each female was recorded. The flowers were brought to the laboratory and cleaned of any plant parts and insects that may have fallen into the collection cylinder. Different whole flower with nectar were offered to *T. brassicae* females in glass tubes (vials). Honey, molasses and the other diets were dotted onto the paper with a sharpened dissecting probe to provide four dots no larger than 2 mm in diameter. Flowers were collected daily and spread on white paper under a lamp to check for insects and then offered to the parasitoids.

Data were analyzed using SPSS 8 Windows. A one-way analysis of variance (ANOVA) was used to study the effects of the food sources applied as a factor and the number of parasitized eggs, adult emergence and female emergence as parasitization efficiency dependent. Means were compared Duncan's Multiple Range test was applied as a means of separation.

Results

Longevity

The longevity of *T. brassicae* females was influenced by their diet (Table 1). When royal jelly + *E. kuehniella* larvae were given, *T. brassicae* individuals lived for only 1.40 ± 0.50 d. Honey + acacia nectar significantly increased the longevity of females ($P < 0.05$) of *T. brassicae*. Females lived the longest when fed with honey + acacia nectar (17.46 ± 6.52 d), honey + apple syrup (17.20±4.44 d), honey (16.93±3.91 d) and honey + *Paulownia* nectar (16.60 ± 4.54 d).

Fecundity

Diet had a significant effect on fecundity ($P < 0.05$). The percentage of fecundity was significantly greater on honey (106.8 ± 30.26 eggs), honey + acacia nectar (105.4 ± 12.26 eggs) and honey + *Paulownia* nectar (103.13 ± 15.34 eggs), than royal jelly + red tulip nectar (3.33 ± 1.34) eggs) (Table 1). A large variation was found within the diets as regards fecundity. The mean parasitism decreased dramatically, especially for females on fed royal jelly and royal jelly + resin.

Adult emergence

The parasitoid completed development on all diets tested (Table 1). The greatest adult emergence was obtained on royal jelly + red tulip nectar (100%) and royal jelly + *E. kuehniella* larvae (100%).

Female emergence

The numbers of females that emerged were significantly affected by the food given. The numbers of female progeny which emerged were different from the pattern shown in the number of parasitized eggs. The highest female emergence was obtained royal jell and combinations (Table 1).

Table 1. Mean longevity, fecundity and percentage of adult and female emergence of *Trichogramma brassicae* Bezdenko [(X±SD) (min-max)]

Diets	Longevity (d)*	Fecundity*	Adult emergence (%)*	Female emergence (%)*
Honey	16.93 ± 3.91 (9-23) H	106.8 ± 30.26 (55-149) L	98.86 ± 2.35 (91-100) BCD	85.00 ± 3.81 (78-95) CD
Molasses	11.60 ± 5.17 (4-22) G	91.60 ± 24.03 (55-131) KL	97.93 ± 2.76 (92-100) ABC	85.13 ± 2.87 (80-90) CD
Royal jelly	3.00 ± 0.92 (2-5) CD	7.40 ± 4.5 (2-15) AB	97.20 ± 5.63 (80-100) ABCD	89.53 ± 10.84 (73-100) F
Honey + acacia nectar	17.46 ± 6.52 (7-26) H	105.40 ± 12.26 (78-121) L	99.33 ± 1.04 (97-100) BCD	85.46 ± 1.92 (83-89) CD
Honey + *Paulownia* nectar	16.60 ± 4.54 (8-23) H	103.13 ± 15.34 (79-126) L	99.26 ± 1.16 (97-100) BCD	83.60 ± 3.56 (75-89) C
Honey + apple syrup	17.20 ± 4.44 (10-24) H	80.20 ± 19.84 (36-110) JK	98.33 ± 1.49 (95-100) ABC	83.00 ± 3.22 (78-90) C
Honey + yellow asphodel nectar	7.66 ± 3.69 (4-17) F	67.93 ± 40.70 (28-151) HI	98.13 ± 1.80 (95-100) ABC	79.13 ± 5.48 (71-86) BC
Honey + red tulip nectar	14.60 ± 3.83 (8-19) H	74.80 ± 29.11 (33-125) IJ	99.06 ± 1.43 (96-100) BCD	80.53 ± 2.87 (75-86) C
Honey + *E. kuehniella* larvae	6.66 ± 2.49 (4-11) EF	51.93 ± 23.01 (23-97) G	98.06 ± 1.98 (94-100) ABC	79.20 ± 6.57 (65-87) BC
Honey + *E. kuehniella* eggs	9.66 ± 2.55 (5-14) G	74.40 ± 19.87 (32-97) IJ	98.93 ± 1.53 (96-100) BCD	84.93 ± 4.78 (72-89) CD
Honey + resin	10.13 ± 2.13 (6-13) G	57.66 ± 13.60 (39-89) GH	98.06 ± 1.83 (96-100) ABC	81.93 ± 2.40 (78-85) C
Molasses + yellow asphodel nectar	7.00 ± 1.30 (5-10) EF	32.20 ± 10.59 (16-59) F	98.53 ± 3.06 (89-100) BCD	69.20 ± 3.66 (63-75) A
Molasses + red tulip nectar	5.73 ± 1.90 (2-9) E	29.73 ± 16.28 (12-66) F	98.40 ± 3.92 (86-100) BCD	70.26 ± 4.78 (57-77) AB
Molasses + apple syrup	10.13 ± 2.66 (5-13) G	65.73 ± 19.07 (37-100) IJ	99.53 ± 1.24 (96-100) CD	80.73 ± 3.41 (75-86) C
Molasses + *Paulownia* nectar	9.93 ± 2.18 (6-13) G	56.60 ±13.38 (38-89) GH	99.20 ± 1.69 (95-100) CD	78.86 ±3.92 (71-84) BC
Molasses + Acacia Nectar	5.60 ± 2.16 (3-10) E	36.26 ± 18.22 (7-77) F	98.26 ± 2.08 (94-100) ABCD	78.26 ± 7.78 (67-100) BC
Molasses + *E. kuehniella* larvae	3.06 ± 1.16 (2-5) CD	16.86 ± 8.45 (7-37) DC	96.06 ± 4.21 (87-100) AB	83.00 ± 6.64 (73-100) C
Molasses + *E. kuehniella* eggs	5.60 ± 2.09 (2-9) E	23.80 ± 7.39 (9-33) EF	97.00 ± 4.14 (86-100) ABC	77.73 ± 5.50 (67-83) ABC
Molasses + resin	3.53 ± 1.18 (2-6) CD	12.80 ± 5.01 (5-23) BC	94.73 ± 5.92 (85-100) A	83.60 ± 10.28 (70-100) CD
Royal jelly + *Paulownia* nectar	3.06 ± 0.70 (2-4) CD	13.13 ± 6.94 (6-34) BC	97.00 ± 5.25 (87-100) ABCD	80.40 ± 7.56 (67-100) C
Royal jelly + red tulip nectar	2.40 ± 0.50 (2-3) BC	3.33 ± 1.34 (2-7) A	100.0 ± 0.00 (100-100) D	97.73 ± 6.08 (80-100) G

Table 1. (Continued)

Diets	Longevity (d)*	Fecundity*	Adult emergence (%)*	Female emergence (%)*
Royal jelly + acacia nectar	3.66 ± 0.72 (2-5) D	20.00 ± 8.16 (11-46) DE	98.00 ± 2.64 (93-100) ABCD	94.20 ± 4.17 (88-100) EF
Royal jelly + resin	2.93 ± 0.70 (2-4) CD	9.53 ± 4.30 (3-18) B	97.80 ± 4.84 (86-100) BCD	96.46 ± 4.58 (88-100) G
Royal jelly + apple syrup	3.06 ± 0.88 (2-5) CD	9.06 ± 4.80 (3-21) B	97.73 ± 4.11 (88-100) ABCD	97.20 ± 4.87 (87-100) G
Royal jelly + *E. kuehniella* larvae	1.40 ± 0.50 (1-2) A	8.86 ± 3.75 (5-18) B	100.0 ± 0.00 (100-100) D	97.60 ± 4.18 (89-100) G
Royal jelly + *E. kuehniella* eggs	1.86 ± 0.74 (1-3) AB	9.26 ± 3.51 (4-16) B	96.46 ± 4.59 (88-100) ABC	89.60 ± 9.25 (71-100) F
Royal jelly + yellow asphodel nectar	1.93 ± 1.03 (1-4) AB	11.13 ± 6.17 (3-21) BC	96.46 ± 4.77 (88-100) ABC	83.26 ± 12.15 (50-100) CD

* Mean in a column the same letters are not significantly different (P<0.05)

Discussion

All natural floral nectars and artificial diets allowed *T. brassicae* to complete development. This indicates that there is a potential for rearing this parasitoid on artificial and floral nectars. Fecundity of *T. brassicae* females was greater honey, honey + acacia nectar and honey + *Paulownia* poplar nectar and this value were significantly higher from those obtained on others. The number of parasitization was lowest royal jelly + red tulip nectar. Cruden & Hermann (1983) found that cut flowers may produce less nectar that intact flowers. Our results correspond with the finding of (Witting-Bissinger, 2008) where *Trichogramma exiguum* Pinto & Platner, 1978 longevity and fecundity were increased significantly when wasps were provided honey and honey + buckwheat. Our results are supported by other studies which found and increase in longevity and fecundity with honey or flowers as a food sources compared with water or no food (Özkan, 2007; Tunçbilek et al., 2012; Çınar et al., 2015).

In this study, with honey as a food source, mean female longevity was ten times longer, and the mean fecundity was 100 times greater than with royal jelly. A number of studies have shown that an increase in fecundity can occur when parasitoid wasps are provided particular food (Leius, 1961; Ashley & Gonzalez, 1974; Yu et al., 1984; Leatemia et al., 1995; Aydin Özder & Kılınçer, 1996 a, b; Blanche et al., 1996; Gurr & Nicol, 2000; Johanowicz & Mitchell, 2000; Costamagna & Landis, 2004; Shearrer & Atanassov, 2004; Fuchsberg et al., 2007; Özkan, 2007; Tunçbilek et al., 2012; Lessard & Boivin, 2013; Çınar et al., 2015). Saljoqi & Khattak (2007) reported that adult *Trichogramma chilonis* Ishii, 1941 females provided with 50% honey and water lived significantly longer than unfed females or those provided with some other kind of food. Feeding has been shown to increase the longevity of *Trichogramma platneri* Nagarkatti, 1975 (Hohmann et al., 1989). Although the availability of honey markedly affected the longevity of *T. platneri,* it did not increase fecundity (Hohmann et al., 1989). Özder et al. (2011) reported that *Trichogramma evanescens* Westwood, 1833 longevity increased significantly when wasps were provided corn pollen + honey compared to pollen alone. In other studies, the mean fecundity of mated females fed with was similar to unfed females (Hohmann et al., 1989, Özder, 2006; Özder & Kara, 2010).

Nectar is a good food for insects, and nectar and pollen have positive effects on longevity, fecundity, adult emergence and female emergence (Lewis & Takasu, 1990; Wäckers, 1994; Patt et al., 1999; Thompson & Hagen, 1999; Wäckers, 2003). Zhang et al. (2004) showed that *T. brassicae* females fed on corn pollen plus water had significantly increased longevity and fecundity compared with those fed on water alone. Also, Zhu et al. (2015) reported that *T. chilonis* females provided *Sesamum indicum* L. flowers lived significantly longer and had significantly increased fecundity than when on provided water.

Trichogrammatid egg parasitoids have been used to reduce egg hatching and subsequent damage caused by larval pests. The success of biological control program may largely depend on potential reproductive rate (the number of adult female progeny produced by a female parasitoid in the presence unlimited prey). In our study, a higher ratio of female to male offspring was observed in *T. brassicae* females provided royal jelly and royal jelly combination. This was probably due to the special food. Royal jelly is a honey bee secretion that is used in nutrition of larvae as well as adult queens and includes water, protein, fat, enzyme, hormone, vitamin and some micronutrients. When worker bees make a new queen, they choose small larvae and feed them with royal jelly. This type of feeding triggers the development of queen morphology including the fully developed ovaries needed to lay eggs (Doğaroğlu & Doğaroğlu, 2015).

Food quality is important in determining the effectiveness of parasitoids as control agents. We conclude that artificial and natural diets are effective for rearing *T. brassicae*, based on parasitization, adult emergence and female longevity. In the current study, artificial diets proved to be suitable foods for sustaining the development and reproduction of *T. brassicae.* Floral nectar qualities may be of importance to parasitoid longevity when selecting floral resources for conservation biological control. Nectar sugar composition may also be crucial in determining its nutritional suitability.

Our results suggest that honey, grape molasses, acacia nectar and *Paulownia* nectar food sources could serve as food sources for *T. brassicae* in stores, warehouses and in the field. While information on the effect of food resources on longevity and reproduction output of parasitoids are important to the study of biological control, additional studies on royal jelly and feeding behavior of parasitoids in the field are needed.

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